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Mycobacterium tuberculosis genotype Beijing: About 15 strains and their part in MDR-TB outbreaks in Algeria

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ABSTRACT

Within the framework of epidemiological surveillance by molecular typing tools conducted in the NRL on multi-resistant strains, the application of spoligotyping on a group of 390 strains consisting of 389 DR-MTB strains and 1 susceptible strain isolated from patients made it possible to detect the presence of 15 strains belonging to the Beijing genotype. All 15 strains were genotyped by MTBDRplus. Among the 15 strains, 11 were typed by RFLP and 9 by MIRU-VNTR.

After analysis of the profiles obtained by the software MIRU-VNTRplus, two spoligotypes (st No. 1 and st No. 265) and four RFLP profiles and a complete identity profile by MIRU-VNTR, information collected on patients allowed the research team to detect a family tie among patients of three different families, as well as one nosocomial TB transmission.

The percentage of Beijing strains found among the patients in this study was 3.8%; this figure does not reflect the reality because it was calculated from an essay on MDR-TB.

To get an idea of the prevalence of Beijing TB strains in this country, a more extensive study is currently being considered.

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Introduction

The distribution of multidrug-resistant tuberculosis (TB) in general is unknown, because the majority of countries are not equipped with diagnostic laboratories and only a minority of laboratories are controlled by the supranational laboratories. The dissemination of strains, especially multidrug-resistant strains, is a real obstacle that compromises both the effectiveness of treatment and the efforts of the programs against TB.

Several epidemics associated with multidrug-resistant strains have been described worldwide, but the most important are those related to the Beijing strains. The Beijing genotype is widespread, especially in Asian countries, where it

represents more than 80% of isolated strains and more than 40% in other countries [1–6].

The power of mutation in the genomic regions involved in the resistance, the rapid evolution of resistance, the low immune response related to their adaptation to the host, compared with other genotypes, gives these strains a particularly virulent character [7]. Therefore, many studies have been conducted towards the identification of factors that promote their dissemination and mechanisms that allow them to adapt to the host and to its defense mechanisms [8].

Since the introduction of a molecular typing tool in the laboratory used by the researchers, the existence of 15 Beijing genotype strains have been identified in a batch of 390 strains of MTB isolated between 1997 and 2011.

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Materials and methods

Materials

Within the framework of an epidemiological survey on multi-drug-resistant tuberculosis (MDR-TB), 389 MDR-TB strains of tuberculosis were studied by spoligotyping. This technique allowed the researchers to identify the existence of 14 Beijing genotype strains. During the course of the research, a strain of MTB genotype Beijing was isolated from a Chinese expatriate in Algeria in a professional framework. This latter strain was susceptible to TB drugs unlike 14 strains isolated in Algerian patients.

Geo-demographic information of patients with Beijing genotype strains such as age at diagnosis, sex, geographical origin or the setting of diagnosis, date of diagnosis, treatment history and outcome of treatment were obtained from the patients' files and the laboratory register, as well as some of the information provided by the treating physician. These data are shown in Table 1.

Methods

Determination of the drug susceptibility

A microscopic examination well as a culture on solid medium was performed on the samples. The study of strain susceptibility to anti-TB drugs was performed by two methods: the proportion method on solid media (Isoniazid, Rifampicin, Ethambutol, Streptomycin, Kanamycin and Ofloxacin) and the molecular biology method as MTBDRplus of HAIN [9,10].

Genotyping of strains

The 15 strains were typed by spoligotyping [11,12]. The RFLP technique [13,14] was performed on 11 strains and the MIRU-VNTR technique [15] has been applied on 9 strains owing to lack of culture material.

Results

Geo-demographic characteristics of patients

Among the 390 strains which were studied by spoligotyping, 15 Beijing genotype strains have been identified. All patients presented with pulmonary TB, including 4 females and 11 males. The age range was between 19 and 58 years.

Of the 15 patients, 2 were cured under treatment (P1,P7), 1 of the 2 in combination with surgical treatment (P1); 4 patients (P6,P10,P13,P14) lost their sight; and the follow-up of the disease was fatal after an average of 2 years for 8 patients (P2,P3,P4,P5,P8,P11,P12,P15) – a 53% death rate.

Regarding the origin of the patients, all patients harboring the Beijing strain were diagnosed in the center region of Algeria covering an area of approximately 200 km; 4 (P10, P12, P13 and P15) are from Dellys, a region located in the province of Tizi Ouzou about 90 km east of Algiers. One patient (P11) is from Miliana, located 60 km south of Algiers. Six patients (P1, P2, P3, P4, P5 and P6) are from Blida, 40 km west of Algiers. Three patients are from Algiers (P7, P8 and P14). The 15th patient (P9) is from China, arrived in Algeria in a professional setting and whose disease was diagnosed in a TB center of Algiers.

Links between the 15 patients

A family tie was found between 3 patients from Blida: P1, P2 and P3. Four other cases were identified among members of two families; one family in Algiers (P7,P8), and the second one in Dellys (P12,P13). A case of nosocomial TB infection (P4) has been identified. It concerns the nurse of the care unit attended by P2.

Drug susceptibility testing

The drug susceptibility testing performed on the 15 strains showed that those isolated in the Algerian patient are resistant to at least RH (study material of investigation MDR-TB).

Table 1 – Geo-demographic data of patients.

Patient No.	Age	Sex	Origin and date of diagnostic	Treatment history	Patient outcome and relationship with other patients of the group
P1	21	F	Blida, 2000	UT	Cured after lobectomy (niece of P2 and P3)
P2	39	M	Blida, 1997	UT	Deceased (uncle of P1)
P3	24	F	Blida, 1998	UT	Deceased
P4	28	F	Blida, 1999	UT	Deceased (nurse in the healthcare center of P2)
P5	42	M	Blida, 2000	UT	Deceased
P6	29	M	Blida, 2002	T	Loss of sight since 2003
P7	22	F	Algiers, 2002	UT	Cured (sister of P8)
P8	24	M	Algiers, 2004	UT	Deceased on post surgical in 2012 (operation on the affected lung)
P9	19	M	China, 2002	UT	Expatriate, returned to China immediately after diagnosis
P10	24	M	Dellys, 2004	UT	Loss of sight since 2005
P11	58	M	Miliana, 2002	T	Deceased
P12	45	M	Dellys, 2004	T	Deceased (brother of P13)
P13	27	M	Dellys, 2007	UT	Loss of sight since 2008
P14	54	M	Algiers, 2003	UT	Loss of sight since 2004
P15	35	M	Dellys, 2001	T	Deceased

H: Isoniazid, S: Streptomycin, R: Rifampicin, E: Ethambutol, M: male, F: female, T: treated, UT: untreated.

Table 2 – Results of sensitivity testing by the proportion method and by the method of MTBDRplus HAIN.

Patient No.	Resistance profiles by the method of proportions	Resistance profile by the method MTBDRplus	Patient No.	Resistance profiles by the method of proportions	Resistance profile by the method MTBDRplus
P1	HSRE	Δ wt7, mut2a (rpoB) Δ wt, mut1 (KatG) Δ wt1, mut1 (inhA)	P9	Susceptible	Susceptible
P2	HSRE	Δ wt7, mut2a (rpoB) Δ wt, mut1 (KatG) Δ wt1, mut1 (inhA)	P10	HSR	Δ Wt8, mut3 (rpoB) Δ Wt, mut1 (KatG) Δ Wt1, mut1
P3	HSRE	wt7, mut2a (rpoB) Δ wt, mut1 (KatG) Δ wt1, mut1 (inhA)	P11	HSR	ND
P4	HSRE	Δ wt7, mut2a (rpoB) Δ wt, mut1 (KatG) Δ wt1, mut1 (inhA)	P12	HSR	Δ Wt8, mut3 (rpoB) Δ Wt, mut1 (KatG) Δ Wt1, mut1 (inhA)
P5	HSRE	wt7, mut2a (rpoB) Δ wt, mut1 (KatG) Δ wt1, mut1 (inhA)	P13	HSR	Wt8, mut3 (rpoB) Δ Wt, mut1 (KatG) Δ Wt1, mut1 (inhA)
P6	HSRE	Δ wt7, mut2a (rpoB) Δ wt, mut1 (KatG) Δ wt1, mut1 (inhA)	P14	HSR	Wt8, mut3 (rpoB) Δ Wt, mut1 (KatG) Δ Wt1, mut1 (inhA)
P7	HSRE	Δ wt7, mut2a (rpoB) Δ wt, mut1 (KatG) Δ wt1, mut1 (inhA)	P15	HSR	ND
P8	HSRE	Δ wt7, mut2a (rpoB) Δ wt, mut1 (KatG) Δ wt1, mut1 (inhA)			

Δ: disappearance of the wildtype probe; mut: mutation probe; ND: not done.

On the other hand, the strain isolated in the Chinese patient is sensitive to TB. Apart from two strains (P11, P15) for which the test MTBDRplus could not be performed, the results of susceptibility testing by the proportion method and by the method of MTBDRplus HAIN are totally concordant and are shown in Table 2.

By the MTBDRplus method, two types of mutations have been found in the *rpoB* gene: the first corresponds to Δ Wt7, mut2a, identified in strains of patients 1, 2, 3, 4, 5, 6, 7, 8 (P1, P2, P3 on the one hand and P7, P8 on the other hand present family tie). The second mutation Δ Wt8, mut3 was identified on strains of patients 10, 12, 13, 14, among them P12 and P13 have a family tie. The mutations found in the *katG* gene and *inhA* gene promoter were identical for the 15 strains.

Molecular typing

Spoligotyping

The profiles of the 390 strains typed by spoligotyping after their comparison to the database SPOLD4 [16] confirmed that 15 strains belong to the Beijing genotype [17].

In this study, of the 15 Beijing strains, 2 spoligotypes were found. The ST1 consists of a cluster of 11 strains and ST 265 consists of a 4-strain cluster; the latter cluster is characterized by the lack of the 37th spacer in addition to the first 34.

RFLP typing

Of the 15 selected Beijing strains, 11 were typed by RFLP. Four different profiles were identified. The number of copies per profile varies between 15 and 16 features attributed to the Beijing genotype strains (Fig. 1). The first 6 profiles (1, 2, 3, 4, 5 and 6 in Fig. 1) are identical and correspond to the group of patients of Blida; the 7th and 8th are the same group of Algiers patients (brother and sister), the 12th and 13th are identical and correspond to the group of Dellys (two brothers), and the last profile (9) belongs to the Chinese patient (single profile). Strains of 4 patients (10, 11, 14 and 15) have not been analyzed by RFLP owing to lack of material.

MIRU-VNTR typing

Among the 15 strains, 9 were typed by 15 MIRU-VNTR (strains of patients 1, 4, 6, 7, 9, 12, 13, 14 and 15). Results of this technique were analyzed by the software MIRU-VNTRplus. They showed that 9 strains have the same number of repetitions for the same MIRU VNTR. The results of the susceptibility testing and the three genotyping techniques are shown in Fig. 1.

Discussion

An epidemiological surveillance essay on the MDR-TB strains by molecular typing tools performed in the researcher's laboratory (spoligotyping, RFLP and MIRU-VNTR) revealed the ge-

Tree	ID	Name	Lineage	Spoligotype	Spoligotype pattern	INH	SM	EMB	RMP	PZA	IS6110	RFLP	MIRU-VNTR
Beijing genotype (H37Rv)	1	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	2	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	3	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	4	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	5	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	6	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	7	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	8	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	9	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	10	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	11	Mt (v)	tuberculosis (v)	1	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
265 genotype	12	Mt (v)	tuberculosis (v)	265	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	13	Mt (v)	tuberculosis (v)	265	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	14	Mt (v)	tuberculosis (v)	265	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3
	15	Mt (v)	tuberculosis (v)	265	r	r	r	r	4 4 2 3 3 3 5 6 4 4 6 5 3 5 3

Fig. 1 – Results of the susceptibility testing and three genotyping techniques, analyzed by the software MIRU-VNTRplus.

netic diversity of strains isolated in Algeria. In addition to the most commonly encountered clades (LAM, H, S), the Beijing genotype was found among the 15 strains isolated from pulmonary TB patients, including 14 Algerians carrying MDR strains and 1 Chinese patient with a drug susceptible strain. These strains were isolated from patients distributed over a geographic area covering approximately 4000 km²: Blida, Algiers, Tizi Ouzou and Miliana.

The analysis of the profiles obtained by spoligotyping has regained two clusters, one of which bears the spoligotype 1 and the other, the spoligotype 265. Typing by RFLP objectified four different profiles. However, the MIRU-VNTR typing showed an identity of profiles between 9 strains typed by MIRU-VNTR 15; by this method, identical results were cited in a study conducted in Benin [18].

The detection of the same genomic profile of strains isolated from patients belonging to the same family and that live in the same geographic area was a clue as to the household as well as the community transmission of tubercle bacilli.

An epidemiological investigation in the field confirmed the existence of a family tie between members of three different families: P1, P2, P3 in Blida, among them, two members are dead and one is cured after surgical treatment in combination with medical treatment. As for patients P7 and P8 from Algiers, one of them was cured after 2 years of treatment and the other died after surgery on the lung. Among members of the family of Dellys, P12 and P13, one of them died after 2 years of treatment. A case of nosocomial TB was diagnosed in a nurse at the TB health center (P4) where her work involved the treatment of TB patients (Table 1).

In a batch of 390 strains analyzed, a Chinese strain susceptible to anti-TB drugs was typed. It turned out that its molecular profile obtained by RFLP is different from those isolated in Algerian patients, confirming the recent importation of this strain in Algeria.

The starting point of this epidemic remains undetermined. The first case diagnosed to be the bearer of the Beijing strain was in 1997 as a result of the introduction of the genotyping methods in the laboratory and their application on multidrug-resistant strains of *Mycobacterium tuberculosis* isolated in pulmonary TB patients.

However, the discovery of strains of the Beijing profile does not surprise the researchers because Algeria has received, particularly during the 1970s, many foreign workers of Asian origin (Chinese and Vietnamese) in the framework of cooperation in the field of health. These were mainly installed in the central region of Algeria, and exercised in the public sector.

The percentage of Beijing strains found in Algeria was 3.8% (15/390 or 14/389 if the recent Chinese patient is excluded). This percentage is overestimated because it was calculated on the Beijing strains isolated from a batch of 389 MDR strains selected as part of a specific study conducted on MDR-TB strains.

A significant bias is up and in any case the percentage reported may not represent the prevalence of Beijing family strains among strains of MTB circulating in the country. It cannot, in this case, be compared with rates reported in multiple studies conducted worldwide on the Beijing genotype of *M. tuberculosis*.

Therefore, an important research is under way to assess the prevalence of the Beijing family strains on a representative sample of strains isolated during a long observation period (1990–2010), without prejudice to the susceptibility or the resistance to anti-tuberculosis drugs.

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